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(54) Title: INTERFERON RECEPTOR BINDING PEPTIDES

(57) Abstract

A polypeptide for use as an interferon receptor-binding peptide, said polypeptide selected from the group of peptides having an amino acid sequence substantially of the formulae; CYS-LEU-LYS-ASP-ARG-HIS-ASP; ASP-GLU-SER-LEU-LEU-GLU-LYS-PHE-TYR-THR-GLU-LEU-TYR-GLN-LEU-ASN-ASP; ASN-GLU-THR-ILE-VAL-GLU-ASN-LEU-LEU-ALA-ASN-VAL-TYR-HIS-GLN-ILE-ASN-HIS; TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA; TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA; TYR-PHE-GLN-TYR; and GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP. The polypeptides are useful for delivering a pharmaceutically active drug to a cell.

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INTERFERON RECEPTOR BINDING PEPTIDES

Background of The Invention

This invention relates generally to receptor binding domains in proteins and more specifically, to specific peptides that interact with the Type 1 human interferon receptor complex.

In order for any pharmaceutical composition to be therapeutically effective, it must be formulated in such a way that it reaches the desired target cells intact. Moreover, once at the site of action, the therapeutic must specifically interact with the target cells. Thus, the design and development of suitable carrier molecules, that may themselves be inert or active, allows for effective targeting of clinically active drugs. Much work has been done in the field of carriers for pharmaceutical compositions. Most recently, peptides have been identified as potentially suitable carriers for pharmaceutical compositions.

The interferons (hereinafter referred to as IFNs) are a family of biologically active proteins that are classified into three major groups, namely, IFN-alpha, IFN-beta and IFN-gamma. IFNs affect a wide variety of cellular functions, related to cell growth control, the regulation of immune responses and more specifically, the induction of antiviral responses. The ability of IFNs to modulate cell growth is observed with many cell types and is particularly effective in the case of tumor cells, which has led to the widespread interest in the use of IFNs for the treatment of neoplaslias.

The presence of a specific receptor at the cell surface is the first requirement for IFN action. Cells that lack these specific receptors are resistant to the effects of IFN. Receptor binding studies have identified the existence of at least two functional IFN receptors that are integral parts of the cell membrane on human cells. Branca, A.A. and Baglioni, C., (1981) Nature 294, 768-770 report that IFN-alpha and IFN-beta bind to one type of receptor and Anderson, P. et

al, (1982) J. Biol. Chem. 257, 11301-11304 report that IFN-gamma binds to a separate receptor. IFN receptors are ubiquitous and more specifically, are upregulated in metabolically active cells such as cancer cells and infected tissues. Although several of the effects of IFNs such as the antiviral state, take several hours to develop, signal transduction immediately following the binding of IFN to its receptor is a rapid event. Since metabolic changes, such as increases in the transcriptional rate of some IFN-induced genes can be detected within five minutes of the addition of IFN, at least some of the transmembrane signals must be very rapid. Hannigan et al, (1986) EMBO J. 5, 1607-1613 suggest that receptor occupancy modulates the transcriptional response of specific genes to IFN. Indeed, there is accumulating evidence to suggest that there is a direct relationship between the number of receptors occupied and the amount of signal that is transduced to the cell nucleus. These transduced signals result in altered gene expression in the nucleus, which mediates the subsequent biological responses.

Extensive studies were undertaken to define those critical clusters of amino acids in the different IFN-alphas and IFN-beta that interact with the Type 1 IFN receptor complex. It is thought that these critical peptide domains would serve as prototypes for synthetic peptides that are useful as carriers for pharmaceutical compositions.

Summary of the Invention

Thus, the present invention is directed to novel peptides which are carriers for pharmaceutical compositions.

More specifically, the invention is directed to novel IFN-receptor binding peptides that are designed as carriers for pharmaceutical compositions.

To this end, in one of its aspects, this invention provides a novel peptide having an amino acid sequence of CYS-LEU-LYS-ASP-ARG-HIS-ASP. (SEQ. ID NO. 1)

In another of its aspects, the invention provides a novel peptide having an amino acid sequence of ASP-GLU-SER-LEU-LEU-GLU-LYS-PHE-TYR-THR-GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP. (SEQ. ID NO. 2)

In still another of its aspects, the invention provides a novel peptide having a sequence of amino acids as follows: ASN-GLU-THR-ILE-VAL-GLU-ASN-LEU-LEU-ALA-ASN-VAL-TYR-HIS-GLN-ILE-ASN-HIS. (SEQ. ID NO. 3)

In another of its aspects, the invention provides a novel peptide having an amino acid sequence of: TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA. (SEQ. ID NO. 4)

The invention also provides a novel peptide having an amino acid sequence of: TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA. (SEQ. ID NO. 5)

A further aspect of the invention is the provision of a novel peptide having an amino acid sequence of: TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR. (SEQ. ID NO. 6)

A still further aspect of the invention is the provision of a novel peptide having an amino acid sequence of: GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP. (SEQ. ID NO. 7)

In yet another of its aspects, the invention provides a pharmaceutical composition which comprises an active drug and a suitable carrier, the carrier having been selected from the group of peptides having an amino acid sequence of CYS-LEU-LYS-ASP-ARG-HIS-ASP (SEQ. ID NO. 1); ASP-GLU-SER-LEU-LEU-GLU-LYS-PHE-TYR-THR-GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 2); ASN-GLU-THR-ILE-VAL-GLU-ASN-LEU-LEU-ALA-ASN-VAL-TYR-HIS-GLN-ILE-ASN-HIS (SEQ. ID NO. 3); TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 4); TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 5); TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR (SEQ. ID NO. 6); and GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 7).

The invention also provides a pharmaceutical composition which comprises an active drug and a suitable carrier, the carrier having been selected from the group of peptides substantially of the formula: CYS-LEU-LYS-ASP-ARG-

HIS-ASP (SEQ. ID NO. 1); ASP-GLU-SER-LEU-LEU-GLU-LYS-PHE-TYR-THR-GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 2); ASN-GLU-THR-ILE-VAL-GLU-ASN-LEU-TYR-ALA-ASN-VAL-VAL-HIS-GLN-ILE-ASN-HIS (SEQ. ID NO. 3); TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 4); TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 5); TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR (SEQ. ID NO. 6); and GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 7).

Brief Description of the Drawings

Figure 1 illustrates the growth inhibitory activities of variant IFN-alphas in T98G cells.

Figure 2 shows five charts illustrating receptor binding characteristics of variant IFN-alphas on T98G cells.

Figure 3 shows four charts illustrating receptor binding characteristics of variant IFN-alphas on T98G cells.

Figure 4 shows secondary structure characteristics of different IFN-alpha species according to amino acid sequence analyses.

Figure 5 is a representation of a model for the tertiary structure of Type 1 IFNs.

Figure Legends

Figure 1

Growth inhibitory activities of variant IFN-αs in T98G cells.

Cells were incubated with the different IFN-α species, at the indicated doses, at 37°C for 96hr, then growth inhibition was estimated by spectrophotometric determination, as described.

Values represent the average of triplicate

determinations and exhibited a SE of ± 4%. □ IFN-α2a; ■ (4-155)IFN-α2a; △ 4-155(S98)IFN-α2a; ▲ 4-155(L98)IFN-α2a; ◇ (ESML)IFN-α2a; ◆ (A30,32,33)IFN-α2a

Figure 2

Receptor binding characteristics of variant IFN-αs on T98G cells.

Binding isotherms. 3.5×10^5 T98G cells were incubated for 2hr at +4°C with the indicated concentrations of ^{125}I-IFN-αCon$_1$, (A), ^{125}I-4-155(S98)IFN-α2a, (B), or ^{125}I-IFN-α1Nδ4, (C). Inset into A, B and C are the corresponding Scatchard plots.

Competitive displacement profiles. 3.5×10^5 T98G cells were incubated at +4°C for 2hr with 10 ng/mℓ ^{125}I-IFN-αCon$_1$, (D), 3.7 ng/mℓ ^{125}I-4-155(S98)IFN-α2a, (E), or 300 ng/mℓ ^{125}I-IFN-α1Nδ4, (F), containing no unlabeled competitor (100% bound) or the indicated concentrations of IFNs.

For D and F: ■ IFN-αCon$_1$; □ IFN-α1Nδ4.

For E: ■ IFN-α2a; □ 4-155(S98)IFN-α2a; △ 4-155(L98)IFN-α2a.

The values shown were obtained by subtracting non-specific counts/min bound from total counts/min bound. Non-specific binding was determined in the presence of a 100-fold excess of unlabeled IFN. The points represent the mean of triplicate cultures and exhibited a S.E. or ± 3%.

Figure 3

Receptor binding characteristics of variant IFN-αs on T98G cells.

Binding isotherms

3.5×10^5 T98G cells were incubated for 2hr at +4°C with the indicated concentrations of ^{125}I-(4-155)IFN-α2a, (A), and ^{125}I-4-155(L98)IFN-α2a, (B). Inset into A and B are the corresponding Scatchard plots.

Competitive displacement profiles

3.5×10^5 T98G cells were incubated at +4°C for 2hr with 20ng/mℓ ^{125}I-(4-155)IFN-α2a, (C), or 8ng/mℓ ^{125}I-4-155(L98)IFN-α2a, (D), containing no unlabeled competitor (100% bound) or the indicated concentrations of IFNs.
■ IFN-α2a; □ (4-155)IFN-α2a; △ 4-155(L98)IFN-α2a; ▲ (ESML)IFN-α2a; ◇ (A30,32,33)IFN-α2a.

The values shown were obtained by subtracting non-specific counts/min bound from total counts/min bound. Non-specific binding was determined in the presence of a 100-fold excess of unlabeled IFN. The points represented the mean of triplicate cultures and exhibited a S.E. of ±3%

Figure 4
Predicted secondary structure characteristics of different IFN-α species according to amino acid sequence analyses.

Hydrophilicity, H, and surface probability, S, profiles are depicted for each of the IFN-αs and IFN-β, whose designations are on the left hand side of each pair. Amino acid residue position is indicated along the horizontal axes of the graphs. The critical domains, comprising residues 29-35, 78-95 and 123-140, are boxed.

Figure 5

Model for the tertiary structure of Type I IFNs.

This model incorporates a helical bundle core, composed of the 5 helices A-E. The loop structures that constitute the proposed receptor recognition epitopes, residues 29-35 and 130-140, shown here as heavily shaded, broad lines, are aligned such that they dock in the receptor groove as shown. The third region implicated in the active conformation of the Type I IFNs, 78-95, is not buried in the receptor groove and is configured to allow binding to its cognate epitope on

another Type 1 IFN receptor. The shaded areas in helices C and D represent residues that are critical for maintaining the correct strucutral presentation of the corresponding contiguous recognition epitopes (see text).

Description of the Preferred Embodiment

Biologically active proteins have an optimum active configuration that is composed of discrete and unique strategic domains along the polypeptide. These critical structural domains determine such parameters as receptor binding and effector functions. Characterization of these strategic domains, that includes defining their spatial configuration and effector functions, will clarify the sequence of events comprising and initiated by receptor binding and that lead to specific biological responses.

For a therapeutic agent to be optimally active, it must be delivered to the specific site of action intact and must interact with the target tissues. In a number of clinical conditions, such as uncontrolled proliferation in neoplastic tissues, or infected tissues, or inflamed tissues, the cells express abundant Type 1 IFN receptors, that is, IFN-alpha and IFN-beta receptor expression at the cell surface is upregulated. It has been determined that specific peptides are capable of recognizing and binding to these cell surface receptors. Once bound, the ligand-IFN receptor complex is transported into the cell.

The present invention relates therefore to novel carriers which comprise peptides of specific amino acid sequences. These sequences are:

(i) an amino acid sequence of CYS-LEU-LYS-ASP-ARG-HIS-ASP (SEQ. ID NO. 1);

(ii) an amino acid sequence of ASP-GLU-SER-LEU-LEU-GLU-LYS-PHE-TYR-THR-GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 2);

(iii) an amino acid sequence of ASN-GLU-THR-ILE-VAL-GLU-ASN-LEU-LEU-ALA-ASN-VAL-TYR-HIS-GLN-ILE-ASN-HIS (SEQ. ID. NO. 3);

(iv) an amino acid sequence of: TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 4);
(v) an amino acid sequence of: TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 5);
(vi) an amino acid sequence of: TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR (SEQ. ID NO. 6); and
(vii) an amino acid sequence of: GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 7).

These novel peptide/carriers have been incorporated into interferons to establish their claimed utility. The following description will be made in conjunction with experiments using interferons having the novel carriers incorporated therein but the invention is not to be restricted to such interferons.

Fish et al in J. IFN Res. (1989) 9, 97-114 have identified three regions in IFN-alpha that contribute toward the active configuration of the molecule. These three regions include: 10-35, 78-107 and 123-166.

The structural homology and symmetry observed among a number of haemopoietic cytokine receptors, and specifically the IFN receptors and tissue factor, the membrane receptor for the coagulation protease factor VII, lends support to the functional receptor binding model that was proposed by Bazan, J.F., Pro. Natl. Acad. Sci. (1990) 87, 6934-6938. This model invokes the presence of a generic binding through that allows recognition of conserved structural elements among different cytokines. The present inventor's data supports such a model, at least for the different IFN-alpha molecular species and IFN-beta, since they have identified two conserved elements in the Type 1 IFNs that effect receptor recognition. A third structural element, that is an exposed recognition epitope, confers specificity of cytokine function, including species specificity.

Experiments were conducted using IFNs shown in Table 1:

TABLE 1

```
                1                   11                  21                  31                  41                  51                  61                  71                  81                  91                 101                 111
IFN-αCon1       CDLPQTHSLG  NRRTLILLAQ  MRRISPFSCL  KDRHDFGFPQ  EEFDGNQFQK  AQAISYLHEM  IQQTFNLFST  KDSSAAWDES  LLEKFYTELY  QQLNDLEACY  IQEVGVEETP  LMNVDSILAV
IFN-α2a         CDLPQTHSLG  SRRTLMLLAQ  MRRISLFSCL  KDRHDFGFPQ  EEF-GNQFQK  AETIPVLHEM  IQQIFNLFST  KDSSAAWDET  LLDKFYTELY  QQLNDLEACY  IQGVGVTETP  LMKEDSILAV
(4-155)IFN-α2a      QTHSLG  SRRTLMLLAQ  MRRISLFSCL  KDRHDFGFPQ  EEF-GNQFQK  AETIPVLHEM  IQQIFNLFST  KDSSAAWDET  LLDKFYTELY  QQLNDLEACY  IQGVGVTETP  LMKEDSILAV
4-155(S98)IFN-α2a   QTHSLG  SRRTLMLLAQ  MRRISLFSCL  KDRHDFGFPQ  EEF-GNQFQK  AETIPVLHEM  IQQIFNLFST  KDSSAAWDET  LLDKFYTELY  QQLNDLEACY  IQGVGVTETP  LLKEDSILAV
4-155(L98)IFN-α2a   QTHSLG  SRRTLMLLAQ  MRRISLFSCL  KDRHDFGFPQ  EEF-GNQFQK  AETIPVLHEM  IQQIFNLFST  KDSSAAWDET  LLDKFYTELY  QQLNDLEACY  IQGVGVTETP  LLKEDSILAV
(ESML)IFN-α2a       CDLPETHSLG  SRRTLMLLAQ  MRRISLSSCL  MDRHDFGFPQ  EFF-GNQFQK  AETIPVLHEM  IQQIFNLFST  KDSSAAWDET  LLDKFYTELY  QQLNDLEACY  IQGVGVTETP  LMKEDSILAV
(A30,32,33)IFN-α2a  CDLPQTHSLG  SRRTLMLLAQ  MRRISLFSCA  KAAHDFGFPQ  EEF-GNQFQK  AETIPVLHLM  IQQIFNLFST  KDSSAAWDET  LLDKFYTELY  QQLNDLEACY  IQGGVVTETP  LMKEDSILAV
IFN-α1N64           ETHSLD  NRRTLMLLAQ  MSRISPSSCL  MDRHDFGFPQ  EEFDGNQFQK  APAISVHLEL  IQQIFNLFTT  KDSSAAWDED  LLDKFCTELY  QQLNDLEACY  IVENLLANVV  LMNADSILAV
IFN-β               MSYNLLGFLQRSS  NFQCCKLLWQ  LNGRLEYCL  KDRMNFDIPE  EDAALTUYEM  LQNIFAIFRQ  DSSTGWNET  RGKLMSSLHL  IVENLLANVV  HQINHLKTVL  EEKLEKEDFT  RGKLMSSLHL
MuIFN-αCon          CDLPQTHNLR  NKRALTLLVQ  MRRLSPLSCL  KDRKDFGFPQ  EKVDAQQIQK  AQAIPVLSEL  TQQILNIFTS  KDSSAAWNAT  LLDSFCNDLH  QQLNDLQACL  MQEVGVQEPP  LTQEDSLLAV  LLDSFCNDLH  QQLNDLQACL
```

Table 1 (Continued)

	121	131	141	151
IFN-αCon$_1$	RKVFQRITLY	LTEKKYSPCA	WEVVRAEIMR	SFSLSTNLQE
IFN-α2a	RKVFQRITLY	LTEKKYSPCA	WEVVRAEIMR	SFSLSTNLQE
(4-155)IFN-α2a	RKVFQRITLY	LTEKKYSPCA	WEVVRAEIMR	SFSLS
4-155(S98)IFN-α2a	RKVFQRITLY	LTEKKYSPCA	WEVVRAEIMR	SFSLS
4-155(L98)IFN-α2a	RKVFQRITLY	LTEKKYSPCA	WEVVRAEIMR	SFSLS
(ESML)IFN-α2a	RKVFQRITLY	LTEKKYSPCA	WEVVRAEIMR	SFSLSTNLQE
(A30,32,33)IFN-α2a	RKVFQRITLY	LTEKKYSPCA	WEVVRAEIMR	SFSLSTNLQE
IFN-α1N64	KKVFRRITLY	LTEKKYSPCA	WEVVRAEIMR	SFSLSTNLQE
IFN-β	KRVYGRILHY	LKAKEYSHCA	WTIVAVEILR	NFYLINRLTG
MuIFN-αCon	RKVFHRITVV	LREKKHSPCA	WEVVRAEVWR	ALSSSANLLA

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IFN-αCon$_1$	RLRRKE
IFN-α2a	SLRSKE
(4-155)IFN-α2a	
4-155(S98)IFN-α2a	
4-155(L98)IFN-α2a	
(ESML)IFN-α2a	SLRSKE
(A30,32,33)IFN-α2a	SLRSKE
IFN-α1N64	RLRRKE
IFN-β	YLRN
MuIFN-αCon	RLSEEKE

Table 1

The foregoing table illustrates the amino acid sequence alignment of the different Type 1 IFNs. The designation of the various IFNs is shown in the left hand column and the sequence of IFN-beta is aligned with the other IFNs, commencing with residue 4, to achieve the greatest homology. The critical domains comprising residues 29-35, 78-95 and 123-140 are boxed. The letter codes for the amino acids are as follows: A, ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

IFN-alpha2a and the various derivatives were provided by I.C.I. Pharmaceuticals Division of the UK; IFN-alphaCon$_1$ was supplied by Amgen of the USA and IFN-alpha$_1$Nδ4 was supplied by Schering Plough Corp of the USA.

IFN-alpha2a, (4-155)IFN-alpha2a, 4-155(S98)IFN-alpha2a and 4-155(L98)IFN-alpha2a had specific activities of 2×10^8 U/mg protein; (A30,32,33)IFN-alpha2a was inactive in antiviral assays and (ESML)IFN-alpha2a had a specific activity of 7.5×10^6U/mg protein; IFN-alphaCon$_1$ had a specific activity of 3.0×10^9U/mg protein; and IFN-alpha$_1$Nδ4 had a specific activity of 7.1×10^6U/mg protein.

The cell culture used comprised T98G cells which were derived from a human glioblastoma multiforma tumor and which express in culture a number of normal and transformed growth characteristics. These cells may be routinely subcultured as monolayers, in modified minimum essential medium (hereinafter referred to as alpha-MEM), and supplemented with 10% (v/v) fetal calf serum (hereinafter referred to as FCS).

An in vitro assay for antiviral activity was conducted. T98G cells were seeded at a density of 1.5×10^5 /mℓ in 200 $\mu\ell$ alpha-MEM supplemented with 10% FCS in 96-well Microtest (trade mark) II tissues culture plates and treated with dilutions of the IFN preparations for 24 hours. At the

time of virus innoculation, the IFNs were removed and 10^4 PFU EMCV was added to individual wells in 100 $\mu\ell$ alpha-MEM, 2% FCS. After 24 hours, the cells were ethanol (95%) fixed and the extent of EMCV infection was determined by spectrophotometric estimation of viral CPE. The fixed cells were crystal violet (0.1% in 2% ethanol) stained and destained (0.5M NaCl in 50% ethanol), and the inhibition of virus infection was estimated from absorbance measurements at 570 nm using a Microplate (trade mark) Reader MR600 and a calibration of absorbance against cell numbers. IFN titers were determined using a 50% cytopathic end-point and converted to international units using an NIH IFN-alpha standard (Ga 23-901-527).

An in vitro assay for growth inhibitory activity was conducted. T98G cells were seeded in 96-well Microtest II tissue culture plates at a density of $5 \times 10^3/m\ell$ and either innoculated with two-fold serial dilutions of different molecular species of IFN-alpha or left untreated. After incubation, at 37°C. for 96 hours, the cells were ethanol fixed (95%), crystal violet (0.1% in 2% ethanol) stained and destained (0.5M NaCl in 50% ethanol), then growth inhibition was estimated from absorbance measurements of destained cells at 570nm (using a Microplate Reader MR600 and a calibration of absorbance against cell numbers).

The results of these experiments are shown in Figure 1. The values represented are the average of triplicate determinations and exhibited a SE of +/-4%. Whereas IFN-alpha2a, (4-155)IFN-alpha2a, 4-155(S98)IFN-alpha2a and 4-155(L98)IFN-alpha2a demonstrate comparable growth inhibitory activities within the error of the assay, (ESML)IFN-alpha2a and (A30,32,33)IFN-alpha2a do not exhibit antiproliferative activity. Similarly, IFN-alpha$_1$Nδ4 has minimal antiviral activity (7.1×10^6 U/mg protein) and no demonstrable antiproliferative activity over the dose range examined.

The next series of experiments examined IFN-receptor interactions. Labelling was carrier out using ^{125}I using a

solid phase lactoperoxidase method. A 100 μℓ reaction mixture containing 10 μℓ 3% B-D-glucose, 10 μℓ hydrated Enzymo-beads (trade mark) (available from BioRad in California, USA) 2 μCi $Na^{125}I$ and 20 μg HuIFN-alpha in PBS, pH 7.2, was reacted overnight at +4°C. Free ^{125}I was separated from IFN-bound ^{125}I on a 12mℓ Sephadex (trade mark) G-75 column, equilibrated in PBS containing 1mg/mℓ BSA. Iodination caused no detectable loss of antiviral activity. Fractions containing maximum antiviral activity were pooled and contained 95% TCA (10%) precipitable radioactivity.

Sub-confluent cell monolayers were incubated at +4°C. in alpha-MEM containing 2% FCS and indicated concentrations of ^{125}I-IFN-alpha. After 2 hours, the binding medium was aspirated and the cultures were washed twice with ice-cold PBS. The cells were solubilized in 0.5M NaOH and radioactivity counted in a Beckman (trade mark) 5500 *-counter. Specificity of binding was determined in parallel binding assays containing a 100-fold excess of unlabeled growth factor. For competitive experiments, specified amounts of unlabeled competitor were included in the reaction mixture together with radiolabelled ligand.

Specific ^{125}I-IFN-alpha binding data were used to determine receptor numbers and dissociation constants, K_d. With increasing concentrations of ^{125}I-ligand in the cellular binding reactions, respective specific binding activities corresponding to each ^{125}I-ligand concentration was calculated.

In Figure 2, panel A illustrates the results using ^{125}I-IFN-alphaCon$_1$; panel B illustrates the results using ^{125}I-4-155(S98)IFN-alpha2a; and panel C illustrates the results using ^{125}I-IFN-alpha$_1$Nδ4. Inset into panels A, B and C are the corresponding Scatchard plots. The competitive displacement profiles are shown in panels D, E and F using 10 ng/mℓ of ^{125}I-IFN-alphaCon$_1$, 3.7 ng/mℓ of ^{125}I-4-155(S98)IFN-alpha2a and 300 ng/mℓ of ^{125}I-IFN-alpha$_1$Nδ4 respectively, with no unlabeled competitor (100% bound) or the indicated concentrations of IFNs. The values shown were obtained by subtracting non-

specific counts/min bound from total counts/min bound. Non-specific binding was determined in the presence of a 100-fold excess of unlabeled IFN. The points represent the mean of triplicate cultures and exhibited a S.E. of +/-3%.

In Figure 3, panel A illustrates the results using ^{125}I-(4-155)IFN-alpha2a and panel B illustrates the results using ^{125}I-4-155(L98)IFN-alpha2a. Inset into panels A and B are the corresponding Scatchard plots. The competitive displacement profiles are shown in panels C and D using 20 ng/mℓ of ^{125}I-(4-155)IFN-alpha2a and 8 ng/mℓ of ^{125}I-4-155(L98)IFN-alpha2a, with no unlabeled competitor (100% bound) or the indicated concentrations of IFNs. The values shown were obtained by subtracting non-specific counts/min bound from total counts/min bound. Non-specific binding was determined in the presence of a 100-fold excess of unlabeled IFN. The points represent the mean of triplicate cultures and exhibited a S.E. of +/-3%.

Figures 2 and 3 illustrate the steady state receptor binding characteristics of the different IFN-alpha molecular species on T98G cells at +4°C. Specific binding to sub-confluent T98G monolayers is resolved into a biphasic Scatchard plot. This IFN binding heterogeneity has been shown to result from negatively cooperative site-site interactions between the ligand receptors. Analysis of the IFN-alpha2a binding data reveals both high and low affinity binding components, with K_ds of 2-3 × 10^{-11} M and 2-5 × 10^{-9} M, respectively. It was found that ^{125}I(ESML) IFN-alpha2a exhibited no detectable binding activity on proliferating (log phase) T98G cells at +4°C. ^{125}I-IFN-alphaCon$_1$ binding to cells was resolved into high affinity K_d 7.7 × 10^{-12} M) and low affinity (K_d 1.4 × 10^{-9} M) components as shown in figure 2A. Similarly, ^{125}I-4-155(S98)IFN-alpha2a (Figure 2B), ^{125}I(4-155)IFN-alpha2a (Figure 3A) and ^{125}I-4-155(L98)IFN-alpha2a (Figure 3B) exhibited binding heterogeneity on T98G cells, with high and low affinity components comparable to IFN-alpha2a. ^{125}I-IFN-alpha$_1$Nδ4 binding to T98G cells was resolved

into a monophasic Scatchard plot, with a single low affinity binding component of K_d 10^{-7} M (Figure 2C). Indeed, competitive binding studies with either ^{125}I-IFN-alphaCon$_1$ (Figure 2D) or ^{125}I-IFN-alpha$_1$Nδ4 (Figure 2F), confirmed that IFN-alpha$_1$Nδ4 has a weaker affinity for the IFN-alpha receptor on T98G cells than IFN-alphaCon$_1$. Substitution of the cysteine residue at position 98 in IFN-alpha2a with a serine, does not affect the polarity or charge distribution of the side chain at this position (CH_2-SH to CH_2-OH), yet substitution with a leucine residue does introduce an aliphatic side chain and hence alter the polarity (CH_2-SH to CH-$(CH_3)_2$). This alteration in side chain polarity at this residue position is not reflected in altered affinity characteristics for the IFN-alpha receptor (Figure 3B). As would be anticipated, substitution of the cysteine residue at position 98 with serine, did not affect receptor binding characteristics (Figure 2B,E). The data from the competitive binding studies, indicate that the IFN-alpha2a variants (ESML)IFN-alpha2a and (A30,32,33)IFN-alpha2a, are unable to bind to the IFN-alpha receptor (Figure 3C,D).

Since the amino acid sequence dictates the native conformation of a protein, the inventor has ascribed protein structure for the different IFN-alphas and IFN-beta. Receptor recognition epitopes are characteristically hydrophilic and located on the surface of the binding molecule. Generally, sites for molecular recognition in proteins are located in loops or turns, whereas alpha-helices are involved in maintaining the structural integrity of the protein. Close examination of the hydrophilicity and surface probability plots of IFN-alpha2a shows that, in those regions that are critical for the active configuration of IFN-alpha, namely 10-35, 78-107 and 123-166, altering the cysteine at 98 has no effect on these determinants (Figure 4), and indeed, does not affect biological activity (Figure 1).

Figure 4 illustrates predicted secondary structure characteristics of different IFN-alpha species according to

amino acid sequence analyses. Hydrophilicity (H) and surface probability (S) profiles are depicted for each of the IFN-alphas and IFN-beta whose designations are on the left hand side of each pair. Amino acid residue position is indicated along the horizontal axes of the graphs. The critical domains comprising residues 29-35, 78-95 and 123-140 are boxed.

In IFN-alpha2a, in the carboxy-terminal domain there are essentially 3 hydrophilic residue clusters that are likely located on the surface of the molecule (Figure 4). Deletion of the cluster closest to the carboxy-terminus, in (4-155)IFN-alpha2a, has no effect on antiviral specific activity, growth inhibitory activity (Figure 1), or receptor binding characteristics (Figure 3), compared with the full length IFN-alpha2a. Thus, for receptor recognition, the region 155-166 does not influence the active configuration of the previously defined strategic domain 123-166. Interestingly, there are two peaks of hydrophilicity in this carboxy-terminal region, that spans residues 123-140, that correspond to a helical bundle and loop structure. In the human, equine, bovine, ovine, rat and murine IFN-alphas, human and murine IFN-beta, cow trophoblast IFN (TP-1) and horse IFN-omega, all designated Type 1 IFNs, these structural motifs are highly conserved (Figure 4), lending credence to the notion that this carboxy-terminally located domain is critical for receptor recognition for the Type 1 IFNs. The alpha-helical structure, that constitutes residues 123-129, allows the appropriate presentation of the loop structure around residues 130-140, and this loop structure serves as a recognition epitope for receptor binding. This conclusion is consistent with reports that the region that comprises residues 123-136 influences biological activities on human and murine cells. Further examination of the 10-35 domain, reveals a single region that is likely located on the surface of the molecule and contains hydrophilic residues, namely 29-35. Other reports have implicated the amino-terminal region of IFN-alpha, in particular amino acid residue 33, as critical for biological

activity on human and bovine cells. The IFN-alpha2a variants (A30-32,33)IFN-alpha2a and (E5,S27,M31,L59)IFN-alpha2a, that have lost biological activity and receptor binding characteristics, no longer present this cluster of residues near the surface of the molecule, (Figure 4). This region constitutes a loop structure. In IFN-alpha$_1$Nδ4, the amino acid residues that immediately precede the critical 29-35 cluster are different to those in IFN-alpha2a, and thus affect the presentation of this receptor binding epitope somewhat, according to the different predictive algorithms the inventor has employed. The data in Figure 4 suggest that the cluster of hydrophilic residues that do constitute this receptor recognition epitope will be located near the surface of the molecule in IFN-alpha$_1$Nδ4. However, substitution of the lysine residue at position 31 by a methionine residue, affects the configuration of this receptor recognition epitope, thereby affecting the biological effectiveness of IFN-alpha$_1$Nδ4. In the human and murine IFNs, the loop structure that includes residues 29-35, is conserved, yet CLKDRHD is presented as CLKDRMN and NLTYRAD, respectively (see Figure 3). In murine consensus IFN-alpha ,MuIFN-alphaCon, this epitope is conserved as CLKDRKD, where H (histidine) to K (lysine) is a conservative change with respect to side chain group and charge. Considerable sequence homology with the human residues 29-35 is also apparent among the murine, equine, ovine, bovine and rat IFN-alphas, as well as for cow TP-1 and horse IFN-omega. The Type 1 IFNs share conserved receptor recognition epitopes in the 29-35 and 123-140 regions. Some variance is seen in the human and murine IFN-beta in the 29-35 region, although the presentation of this epitope as a loop structure is conserved.

The third strategic region with respect to the active configuration of IFN-alpha spans residues 78-107. A hydrophilic cluster of amino acid residues that are likely located on the surface constitute residues 83-95 (Figure 4). These residues probably present as a contiguous helical bundle

and a loop structure. Several amino acid residues around position 78 also appear to be located at the surface as part of the helical bundle. The inventor has shown that substitution of the cysteine at position 98 with either a serine (S) or a leucine (L) does not affect the receptor binding characteristics of IFN-alpha2a, hence the inventor infers that those residues beyond 95, in the previously defined domain 78-107, are likely not critical for receptor recognition in IFN-alpha, since they appear not to be located at the surface of the molecule. The alpha-helical structure allows the appropriate presentation of the recognition epitope that comprises residues 88-95. Of note is the variance in this region between the human IFN-alphas and the murine IFN-alphas, and the human IFN-alphas had human IFN-beta. Of the three previously defined critical active domains in the Type I IFNs, it is this domain that exhibits the most divergence with respect to species, and alpha-versus beta-IFNs (Table 1). It is noteworthy that the hybrid IFN, IFN-alphaAD(BgI II), exhibits a hydrophilicity plot somewhat different from the human IFN-alphas in this region, yet similar to that seen for the murine IFNs, specifically MuIFN-alphaCon (Figure 4). Both MuIFN-alphaCon and IFN-alphaAD(BgI II) have a cysteine residue at position 86, in contrast with the majority of human IFN-alphas, for which there is a tyrosine residue in this position. These data are consistent with IFN-alphaAD(BgI II) showing demonstrable biological activity on murine cells and support the hypothesis that this region in the Type I IFNs determines species specificity. Indeed, the hybrid IFN-alphaAD(PvuII) resembles the human IFN-alphas in this region (Figure 4) and differs from IFN-alphaAD(BgI II) at just three residue positions, two of which reside in this critical domain: 69 (S/T), 80(T/D) and 86(Y/C). IFN-alphaAD(Pvu II) demonstrates considerably reduced antiviral activity on murine cells compared with IFN-alphaAD(BgI II) yet comparable activity to IFN-alpha 2a, on human cells.

Sequence homology among the different Type 1 IFNs in

conserved regions would suggest evolutionary significance. It is noteworthy that the amino-and carboxy-terminal domains that have been identified as critical, are highly conserved among the different molecular subtypes of Type 1 IFNs. Within the 29-35 and 123-140 regions are structural motifs that are consistent with receptor binding domains: loop structures that are predominantly hydrophilic and located at the surface of the molecule. Some variation in sequence homology is apparent in the 78-95 region. The critical epitopes for Type I IFN receptor recognition are associated with the residue clusters 29-35 and 130-140, for all species of Type I IFNs. These epitopes constitute the receptor binding domains and are likely located in close spacial proximity to one another in the folded IFN. The specificity of action of a particular Type I IFN is conferred by the recognition epitope 78-95.

The basis for the specificity of interaction of the 78-95 domain and its putative cognate binding molecule is unknown. Studies with human growth hormone have shown that receptor binding involves both receptor recognition, by an epitope on the growth hormone, and dimerization of receptors, facilitated through the interaction of a separate epitope on the growth hormone. By analogy, once an IFN-alpha molecule is bound to its receptor, mediated by the recognition epitopes 29-35 and 130-140, the 78-95 epitope in HuIFN-alpha may interact with another Type 1 receptor, effecting dimerization. Using the cross-linking agent disuccinimidyl suberate for analysis of affinity- labeled cellular IFN binding components, the inventor and a number of other groups have shown that IFN-receptor complexes of 80kDa and 140-160kDa can be separated by SDS-PAGE. The molecular weight of the predicted IFN-alpha receptor protein is 63kDa and that of the majority of IFN-alphas is 20kDa, thus, monomer (receptor-IFN) and dimerized-(receptor-IFN-receptor) complexes, may represent the 80kDa and 140-160kDa moieties that have been detected.

Figure 5 illustrates a model for the tertiary structure of Type 1 IFNs. This model incorporates a helical

bundle core, composed of the five helices A to E. The loop structures that constitute the proposed receptor recognition epitopes, residues 29-35 and 130-140, are shown as heavily shaded, broad lines and are aligned such that they dock in the receptor groove as shown. The third region implicated in the active conformation of the Type 1 IFNs, 78-95, is not buried in the receptor groove and is configured to allow binding to its cognate epitope on another Type 1 IFN receptor. The shaded areas in helices C and D represent residues that are critical for maintaining the correct structural presentation of the corresponding contiguous recognition epitopes. In agreement with a number of different models that have been proposed, the Type I IFNs are comprised predominantly of alpha-helical bundles that are packed together. The receptor recognition site is comprised of the AB loop, 29-35 and the D helix and DE loop, 123-140. These are aligned in such a way as to permit the IFN to bind to its receptor, in the receptor groove, such that the third epitope, 78-95, is exposed and not buried in the receptor groove. The initial interaction of the IFN molecule with the Type I IFN receptor would account for the abundant, low affinity receptor binding component, extrapolated from the Scatchard analyses of the different binding isotherms. The higher affinity component could be invoked once the IFN molecule is bound to its receptor. The heterogeneity of binding observed for IFN-alpha2a is absent in IFN-alpha$_1$Nδ4, and is explained by the alteration of the 29-35 and 78-95 epitopes in IFN-alpha$_1$Nδ4, as compared with IFN-alpha2a. This may lead to a reduction in signaling potential of the receptor-bound IFN and hence a reduction in biological potency.

There is some evidence to suggest that the proliferative state of a cell will determine whether the high affinity binding component is invoked on IFN-alpha2a binding to its receptor. Non-proliferating cells express fewer Type I IFN receptors and will not exhibit the characteristic heterogeneity of binding seen with proliferating cells.

Interestingly, non-proliferating cells do possess both the 80kDa and 140-160kDa IFN-binding complexes. The data indicate that non-proliferating cells lack the high affinity component of IFN-alpha binding, that is not associated with IFN-receptor dimerization, yet may represent a secondary binding molecule. A comprehensive binding model, therefore, that would account for heterogeneity of binding distinct from receptor dimerization, would invoke the interaction of the IFN-bound receptor complex with a putative secondary binding molecule. The possibility that other accessory molecules are required for the full complement of IFN-receptor interactions, is supported by observations of high molecular weight complexes containing the IFN-alpha-receptor complex. Furthermore, the genetic transfer of the human IFN-alpha receptor into mouse cells, led to transfectants that exhibited a poor sensitivity to selected Type 1 human IFNs. These results infer that the transfected protein may not be sufficient for the complete binding activities of the IFNs. Indeed, in the receptor systems described for interleukin-6 and nerve growth factor, accessory proteins are required for the high affinity binding component of the receptor-ligand interaction. In the absence of experimental data, it cannot be discounted that the 78-95 epitope in Type 1 IFNs may interact with a species-specific secondary binding molecule. It is intriguing to suggest that the differential specificity of action that resides in IFN-alpha and IFN-beta, results from the specific interaction of the 78-95 region in the two IFNs with a complementary cognate accessory binding molecule. Moreover, the species specificity observed for the Type 1 IFNs may reside in the recognition of this species-specific cognate binding molecule, by the specific and variable 78-95 epitopes amongst the different Type 1 IFN species. The precedent for major determinants of specificity of interaction has been made with small nuclear ribonucleoproteins and specific RNAs: RNA binding specificity is conferred by short stretches of variant amino acid residues in two ribonucleoproteins that otherwise share extensive

sequence homology. Certainly, among DNA binding proteins, exchange of amino acid residues between members of the helix-turn-helix and zinc finger protein families can result in the exchange of DNA binding specificity. The nature of the accessory binding molecule that may be associated with the Type 1 IFN receptor complex remains to be clarified.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: FISH, Eleanor N.

(ii) TITLE OF INVENTION: INTERFERON RECEPTOR BINDING PEPTIDES

(iii) NUMBER OF SEQUENCES: 17

(iv) CORRESPONDENCE ADDRESS:
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(C) CITY: Toronto
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA
(A) APPLICATION NUMBER: PCT
(B) FILING DATE: 06-JUL-1993
(C) CLASSIFICATION:

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FILING DATE: 07-JUL-1992
(B) APPLICATION NUMBER: US 07/980,525
FILING DATE: 20-NOV-1992

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Cys Leu Lys Asp Arg His Asp
1 5

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Asp Glu Ser Leu Leu Glu Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu
1 5 10 15

Asn Asp

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Asn Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile
1 5 10 15

Asn His

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro
1 5 10 15

Cys Ala

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Tyr Phe Gln Arg Ile Thr Leu Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Glu Leu Tyr Gln Gln Leu Asn Asp
1 5

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 166 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Thr Leu Ile
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Pro Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe
35 40 45

Gln Lys Ala Gln Ala Ile Ser Tyr Leu His Glu Met Ile Gln Gln Thr
50 55 60

Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Ser
65 70 75 80

Leu Leu Glu Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
85 90 95

Glu Ala Cys Tyr Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu Met
100 105 110

Asn Val Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
115 120 125

Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
130 135 140

Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
145 150 155 160

Arg Leu Arg Arg Lys Glu
165

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 166 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Xaa Gly Asn Gln Phe
35 40 45

Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile
50 55 60

Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
65 70 75 80

Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
85 90 95

Glu Ala Cys Tyr Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met
100 105 110

Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
115 120 125

Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
130 135 140

Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
145 150 155 160

Ser Leu Arg Ser Lys Glu
165

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(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 150 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met Leu Leu Ala Gln
1 5 10 15

Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp Arg His Asp Phe
20 25 30

Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr
35 40 45

Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser
50 55 60

Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe
65 70 75 80

Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Tyr Ile
85 90 95

Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys Glu Asp Ser Ile
100 105 110

Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu
115 120 125

Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met
130 135 140

Arg Ser Phe Ser Leu Ser
145 150

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 150 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met Leu Leu Ala Gln
1 5 10 15

Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp Arg His Asp Phe
20 25 30

Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr
35 40 45

Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser
50 55 60

Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe
65 70 75 80

Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Tyr Ile
85 90 95

Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys Glu Asp Ser Ile
100 105 110

Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu
115 120 125

Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met
130 135 140

Arg Ser Phe Ser Leu Ser
145 150

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 150 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met Leu Leu Ala Gln
1 5 10 15

Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp Arg His Asp Phe
20 25 30

Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr
35 40 45

Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser
50 55 60

Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe
65 70 75 80

Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Tyr Ile
85 90 95

Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys Glu Asp Ser Ile
100 105 110

Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu
115 120 125

Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met
130 135 140

Arg Ser Phe Ser Leu Ser
145 150

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 165 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Cys Asp Leu Pro Glu Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Ser Ser Cys Leu Met Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Leu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Tyr Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 165 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Cys Asp Leu Pro Glu Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Ala Lys Ala
20 25 30

Ala His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Leu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Tyr Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 162 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Glu Thr His Ser Leu Asp Asn Arg Arg Thr Leu Met Leu Leu Ala Gln
1 5 10 15

Met Ser Arg Ile Ser Pro Ser Ser Cys Leu Met Asp Arg His Asp Phe
20 25 30

Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Pro
35 40 45

Ala Ile Ser Val His Leu Glu Leu Ile Gln Gln Ile Phe Asn Leu Phe
50 55 60

Thr Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Asp Leu Leu Asp Lys
65 70 75 80

Phe Cys Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Tyr
85 90 95

Met Gln Glu Glu Arg Val Gly Glu Thr Pro Leu Met Asn Ala Asp Ser
100 105 110

Ile Leu Ala Val Lys Lys Tyr Phe Arg Arg Ile Thr Leu Tyr Leu Thr
115 120 125

Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile
130 135 140

Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Arg Leu Arg Arg
145 150 155 160

Lys Glu

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 166 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
1 5 10 15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
20 25 30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Glu Lys Gln Leu Gln
35 40 45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
50 55 60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65 70 75 80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Val His Gln Asn His
85 90 95

Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr Phe
100 105 110

Ile Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
115 120 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
130 135 140

Ile Val Ala Val Glu Ile Leu Arg Asn Phe Tyr Leu Ile Asn Arg Leu
145 150 155 160

Thr Gly Tyr Leu Arg Asn
165

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 168 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Cys Asp Leu Pro Gln Thr His Asn Leu Arg Asn Lys Arg Ala Leu Thr
1 5 10 15

Leu Leu Val Gln Met Arg Arg Leu Ser Pro Leu Ser Cys Leu Lys Asp
20 25 30

Arg Lys Asp Phe Gly Phe Pro Gln Glu Lys Val Asp Ala Gln Gln Ile
35 40 45

Gln Lys Ala Gln Ala Ile Pro Val Leu Ser Glu Leu Thr Gln Gln Ile
50 55 60

Leu Asn Ile Phe Thr Ser Lys Asp Ser Ser Ala Ala Trp Asn Ala Thr
65 70 75 80

Leu Leu Asp Ser Phe Cys Asn Asp Leu His Gln Cys Leu Asn Asp Leu
85 90 95

Gln Ala Cys Leu Met Gln Glu Val Gly Val Gln Glu Pro Pro Leu Thr
100 105 110

Gln Glu Asp Ser Leu Leu Ala Val Arg Lys Tyr Phe His Arg Ile Thr
115 120 125

Val Val Leu Arg Glu Lys Lys His Ser Pro Cys Ala Trp Glu Val Val
130 135 140

Arg Ala Glu Val Val Val Arg Ala Leu Ser Ser Ser Ala Asn Leu Leu
145 150 155 160

Ala Arg Leu Ser Glu Glu Lys Glu
165

I claim:

1. A novel IFN-receptor binding peptide having an amino acid sequence substantially of the formula: CYS-LEU-LYS-ASP-ARG-HIS-ASP (SEQ. ID NO. 1).

2. A novel IFN-receptor binding peptide having an amino acid sequence substantially of the formula: ASP-GLU-SER-LEU-LEU-GLU-LYS-PHE-TYR-THR-GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 2).

3. A novel IFN-receptor binding peptide having an amino acid sequence substantially of the formula: ASN-GLU-THR-ILE-VAL-GLU-ASN-LEU-LEU-ALA-ASN-VAL-TYR-HIS-GLN-ILE-ASN-HIS (SEQ. ID NO. 3).

4. A novel IFN-receptor binding peptide having an amino acid sequence substantially of the formula. TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 4).

5. A novel IFN-receptor binding peptide having an amino acid sequence substantially of the formula: TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 5).

6. A novel IFN-receptor binding peptide having an amino acid sequence substantially of the formula: TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR (SEQ. ID NO. 6).

7. A novel IFN-receptor binding peptide having an amino acid sequence substantially of the formula: GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 7).

8. A pharmaceutical composition comprising an active ingredient and a carrier, said carrier comprising the peptide as claimed in claim 1.

9. A pharmaceutical composition comprising an active ingredient and a carrier, said carrier comprising the peptide as claimed in claim 2.

10. A pharmaceutical composition comprising an active ingredient and a carrier, said carrier comprising the peptide as claimed in claim 3.

11. A pharmaceutical composition comprising an active ingredient and a carrier, said carrier comprising the peptide as claimed in claim 4.

12. A pharmaceutical composition comprising an active ingredient and a carrier, said carrier comprising the peptide as claimed in claim 5.

13. A pharmaceutical composition comprising an active ingredient and a carrier, said carrier comprising the peptide as claimed in claim 6.

14. A pharmaceutical composition comprising an active ingredient and a carrier, said carrier comprising the peptide as claimed in claim 7.

15. A pharmaceutical composition which comprises an active drug and a suitable carrier, the carrier having been selected from the group of peptides having an amino acid sequence substantially of the formulae: CYS-LEU-LYS-ASP-ARG-HIS-ASP (SEQ. ID NO. 1); ASP-GLU-SER-LEU-LEU-GLU-LYS-PHE-TYR-THR-GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 2); ASN-GLU-THR-ILE-VAL-GLU-ASN-LEU-LEU-ALA-ASN-VAL-TYR-HIS-GLN-ILE-ASN-HIS (SEQ. ID NO. 3); TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 4); TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 5); TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR (SEQ. ID NO. 6); and GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 7).

16. A polypeptide for use as an interferon-binding peptide, said polypeptide selected from the group of peptides having an amino acid sequence substantially of the formulae: CYS-LEU-LYS-ASP-ARG-HIS-ASP (SEQ. ID NO. 1); ASP-GLU-SER-LEU-LEU-GLU-LYS-PHE-TYR-THR-GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 2); ASN-GLU-THR-ILE-VAL-GLU-ASN-LEU-LEU-ALA-ASN-VAL-TYR-HIS-GLN-ILE-ASN-HIS (SEQ. ID NO. 3); TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 4); TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 5); TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR (SEQ. ID NO. 6); and GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 7).

17. A method of delivering a pharmaceutically active drug to a cell, which method comprises mixing said drug with a carrier having been selected from the group of peptides having an amino acid sequence substantially of the formulae: CYS-LEU-LYS-ASP-ARG-HIS-ASP (SEQ. ID NO. 1); ASP-GLU-SER-LEU-LEU-GLU-LYS-PHE-TYR-THR-GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 2); ASN-GLU-THR-ILE-VAL-GLU-ASN-LEU-LEU-ALA-ASN-VAL-TYR-HIS-GLN-ILE-ASN-HIS (SEQ. ID NO. 3); TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 4); TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 5); TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR (SEQ. ID NO. 6); and GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 7) and binding said drug and carrier to said cell.

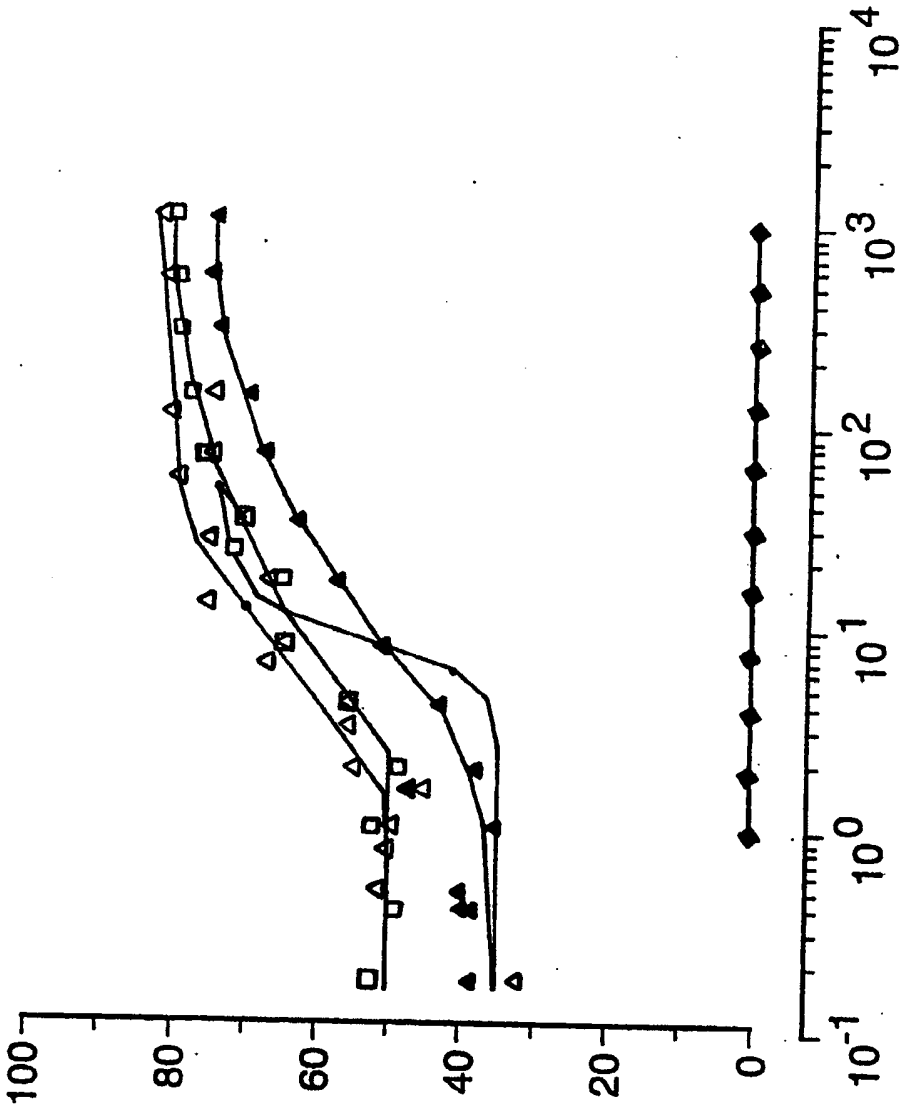
100
80
60
40
20
0
percent growth inhibition
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
10^{4}
ng / ml

FIG.1.

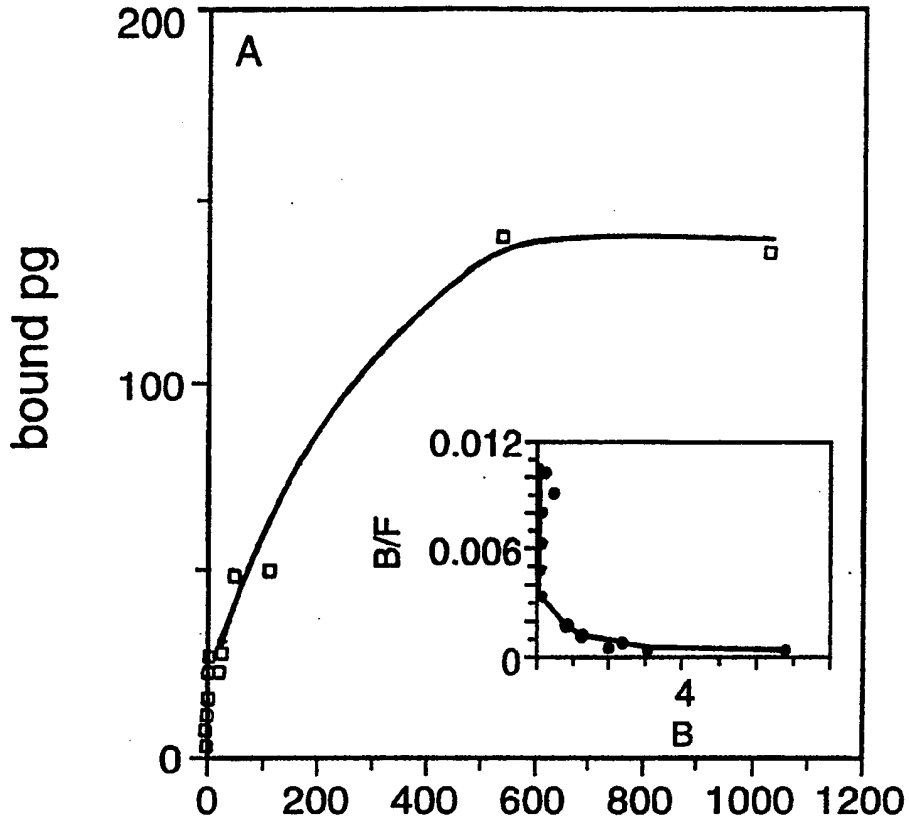
A
bound pg
200
100
0
0
200
400
600
800
1000
1200
B/F
0.012
0.006
0
4
B

FIG.2A.

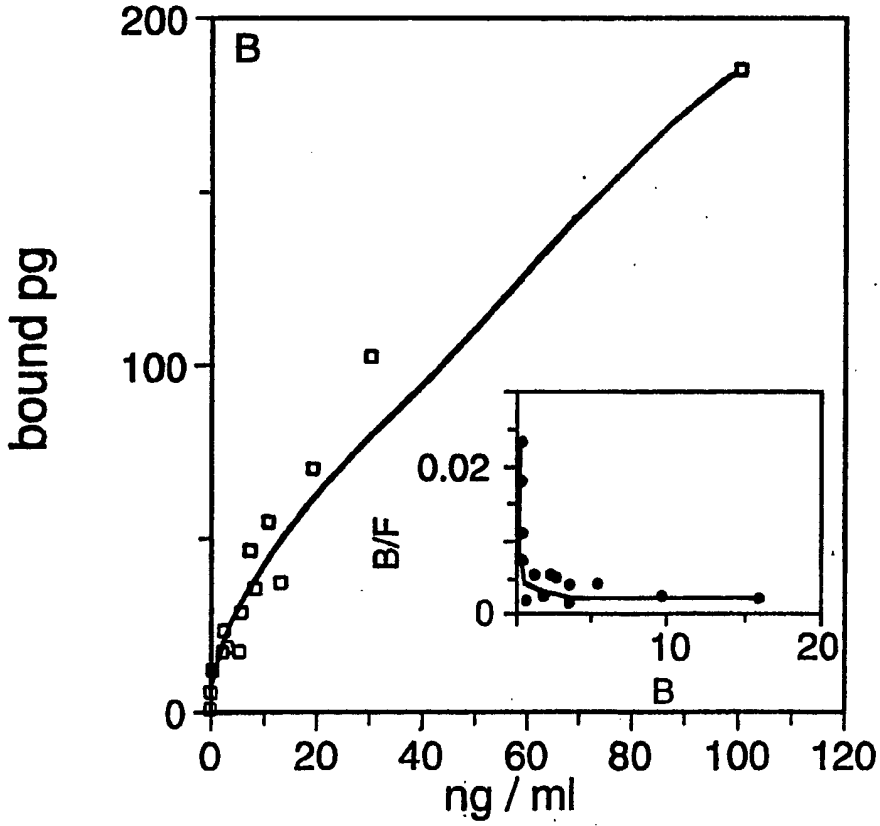
B
bound pg
200
100
0
0
20
40
60
80
100
120
ng / ml
B/F
0.02
0
10
20
B

FIG.2B.

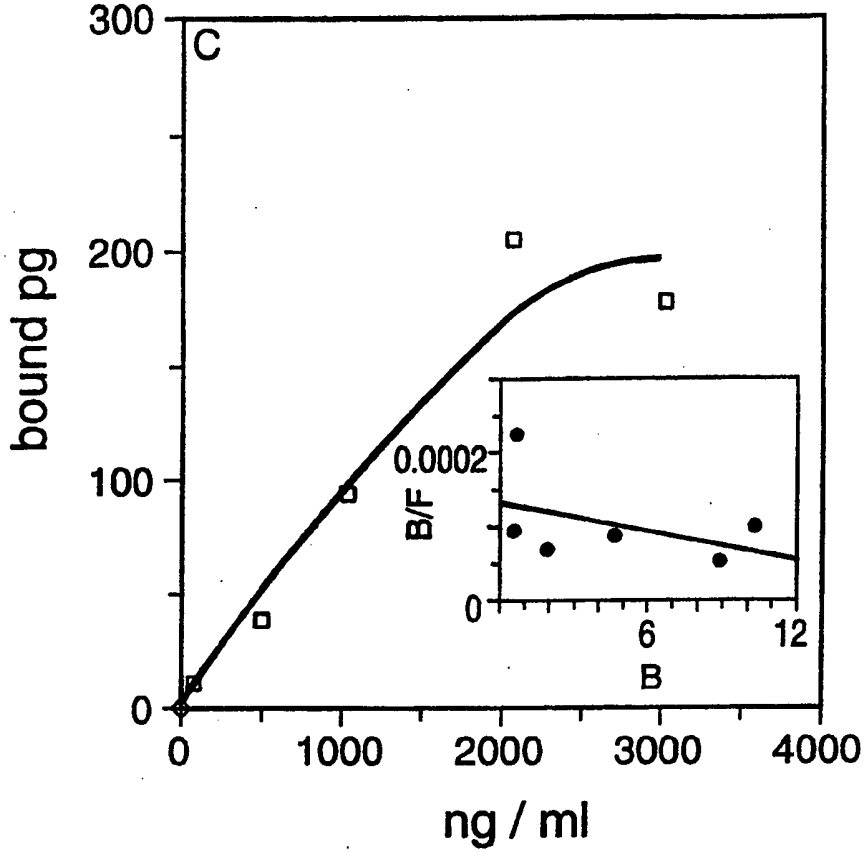
C
300
200
100
0
bound pg
0
1000
2000
3000
4000
ng / ml
0.0002
B/F
0
6
12
B

FIG.2C.

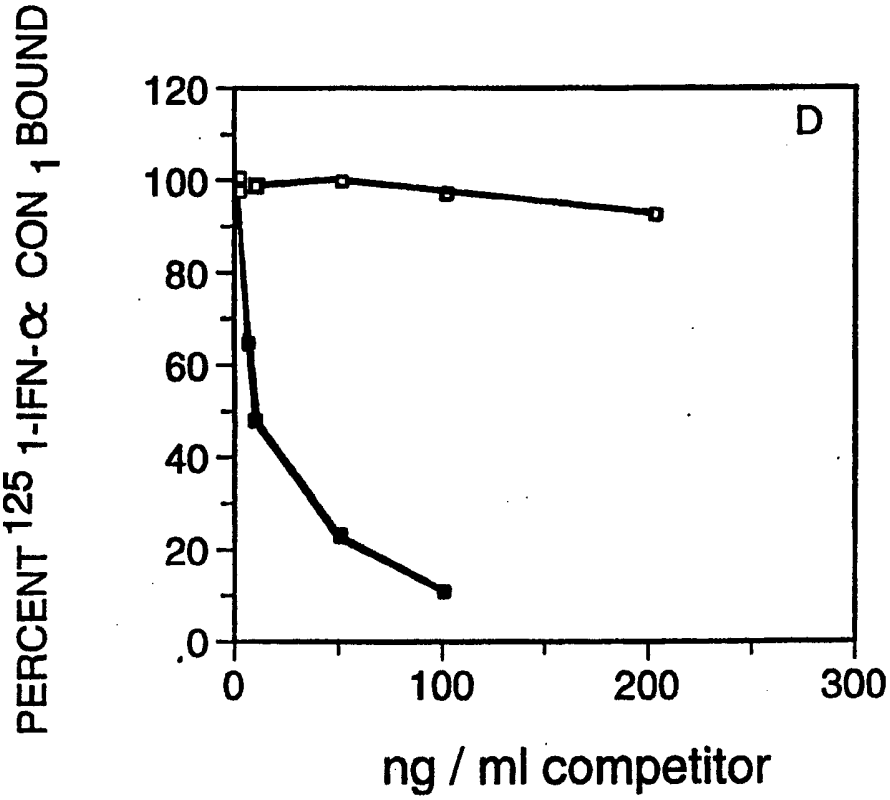
D
120
100
80
60
40
20
.0
PERCENT 125 1-IFN-α CON 1 BOUND
0
100
200
300
ng / ml competitor

FIG.2D.

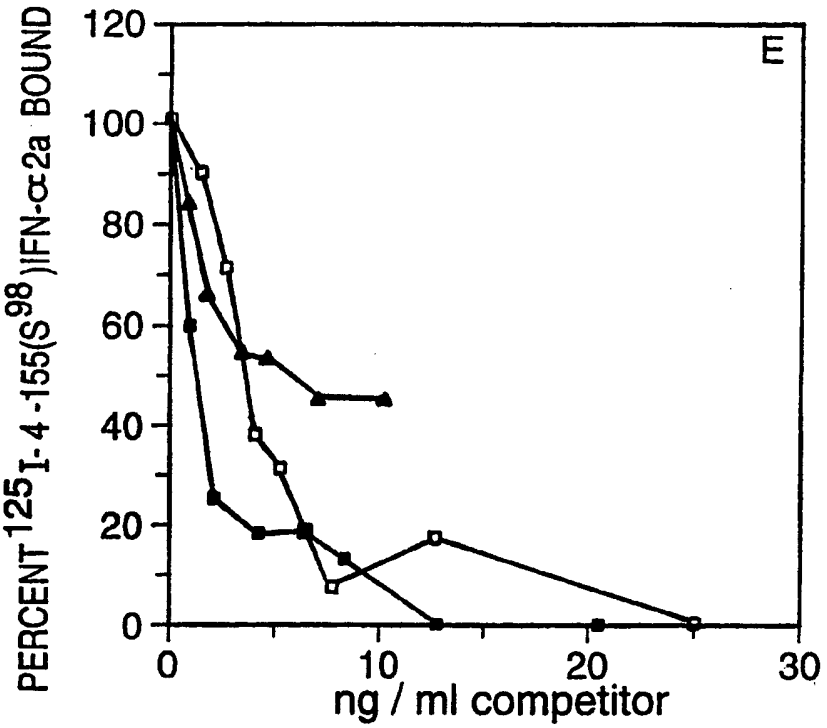
E
PERCENT ^{125}I-4-155(S^{98})IFN-α2a BOUND
120
100
80
60
40
20
0
0
10
20
30
ng / ml competitor

FIG.2E.

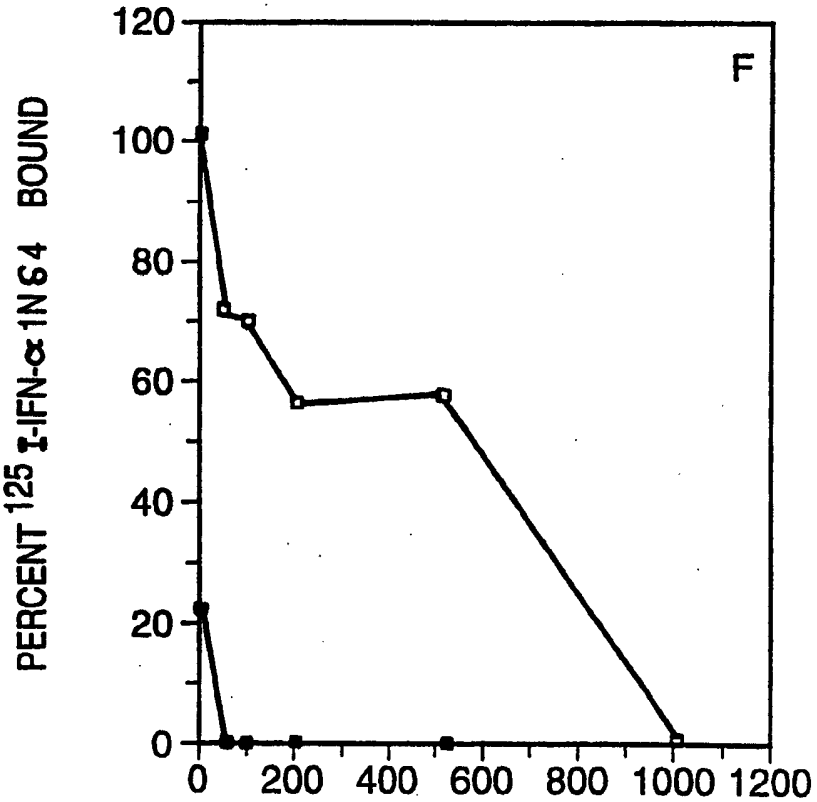
F
PERCENT ^{125}I-IFN-α1N64 BOUND
120
100
80
60
40
20
0
0
200
400
600
800
1000
1200
ng / ml competitor

FIG.2F.

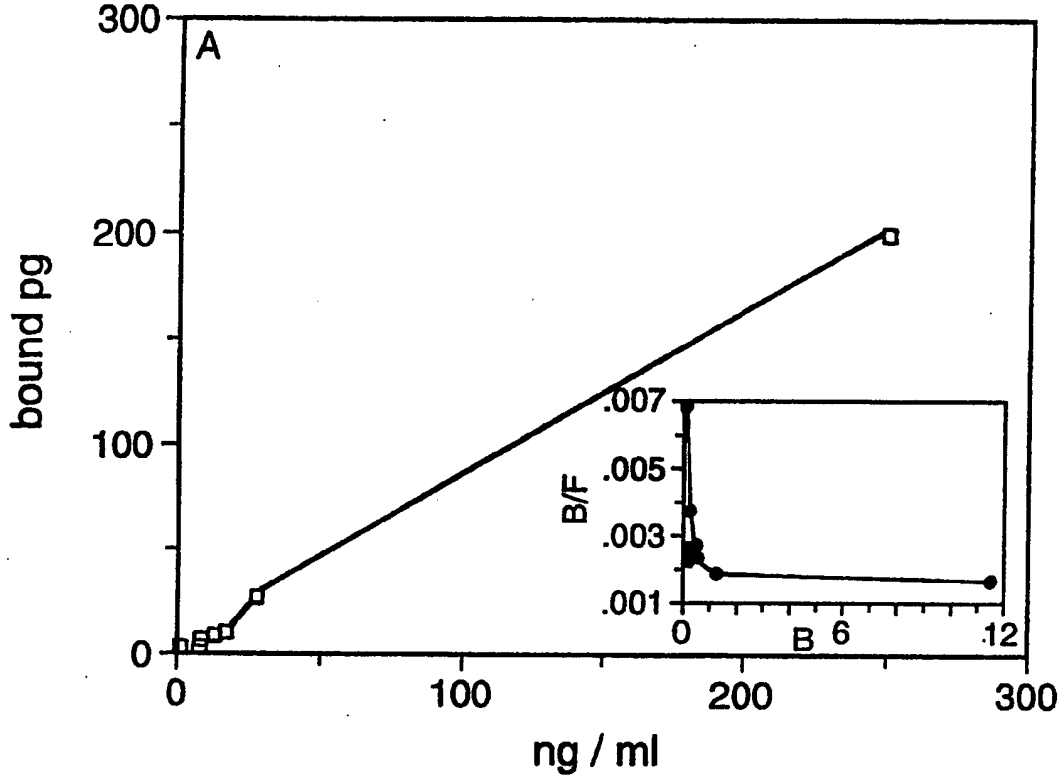
A
bound pg
ng / ml
B/F
B
0
100
200
300
.001
.003
.005
.007
6
12

FIG.3A.

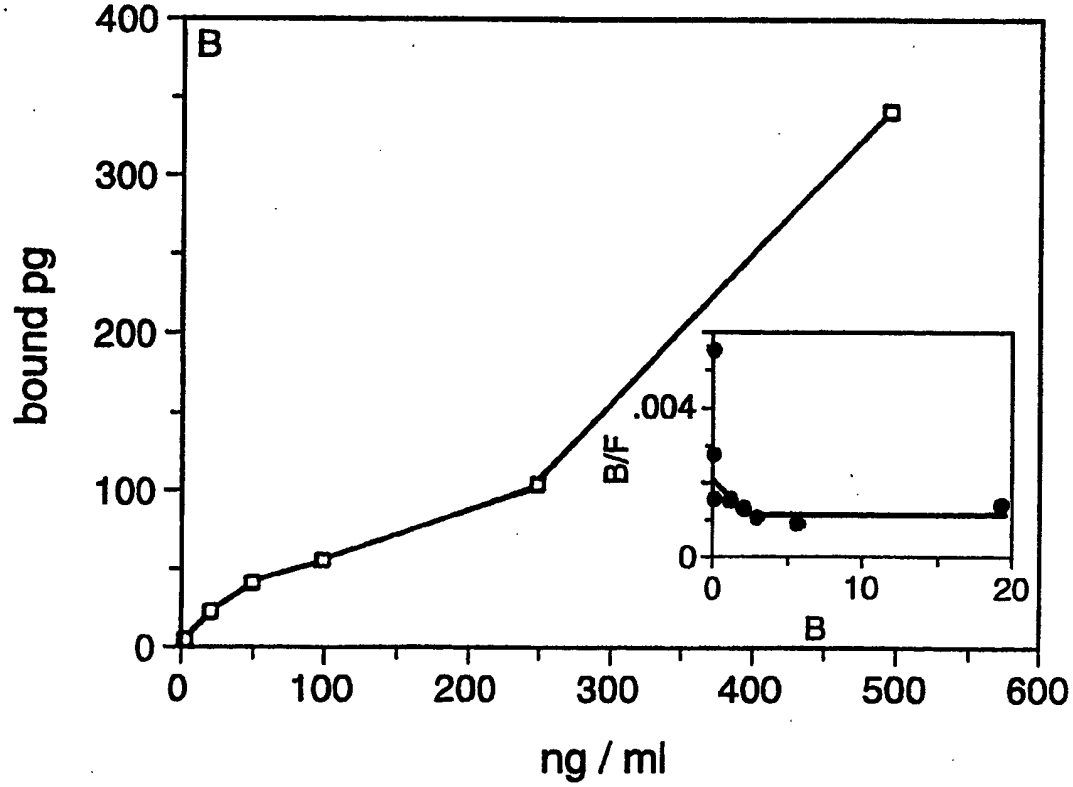
B
bound pg
ng / ml
B/F
B
0
100
200
300
400
500
600
.004
10
20

FIG.3B.

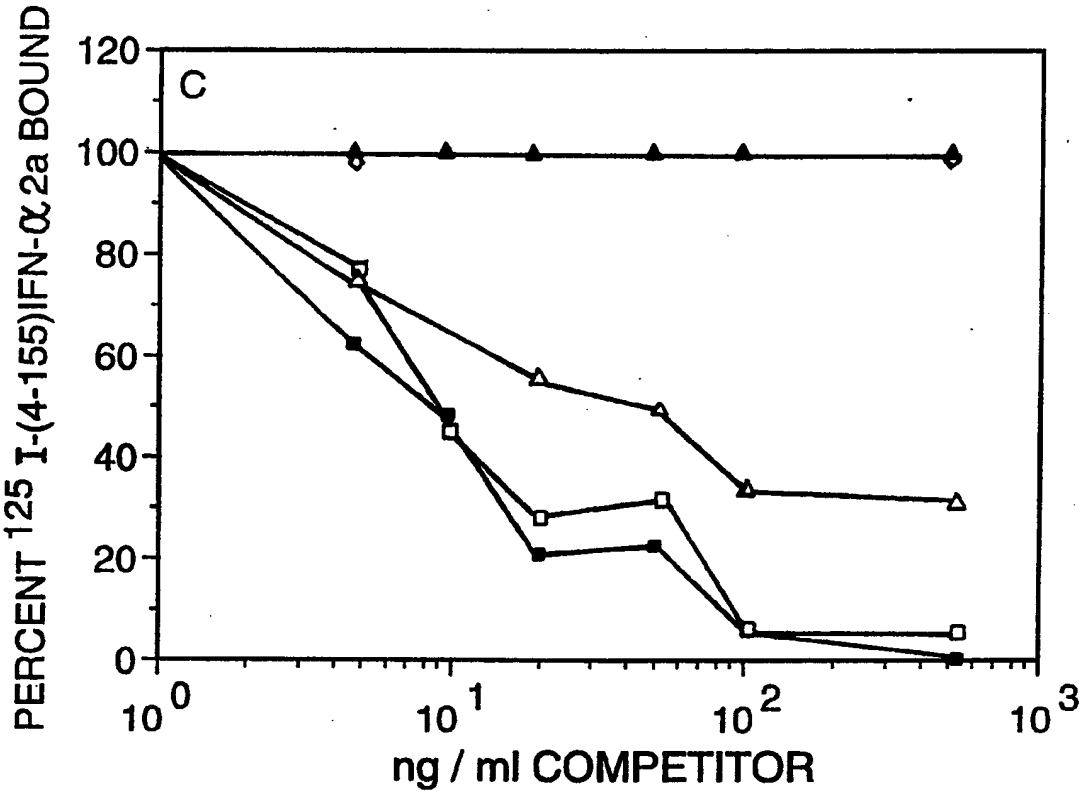
C
PERCENT 125 I-(4-155)IFN-α 2a BOUND
120
100
80
60
40
20
0
10 0
10 1
10 2
10 3
ng / ml COMPETITOR

FIG.3C.

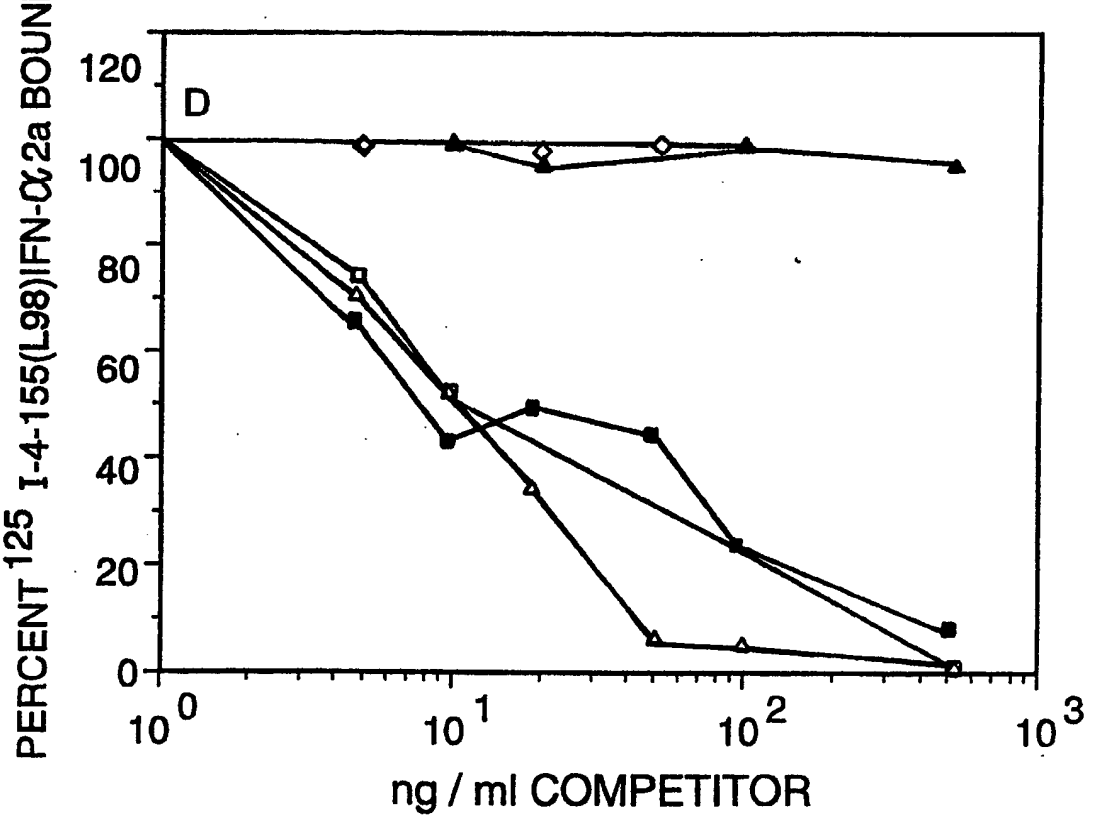
D
PERCENT 125 I-4-155(L98)IFN-α 2a BOUND
120
100
80
60
40
20
0
10 0
10 1
10 2
10 3
ng / ml COMPETITOR

FIG.3D.

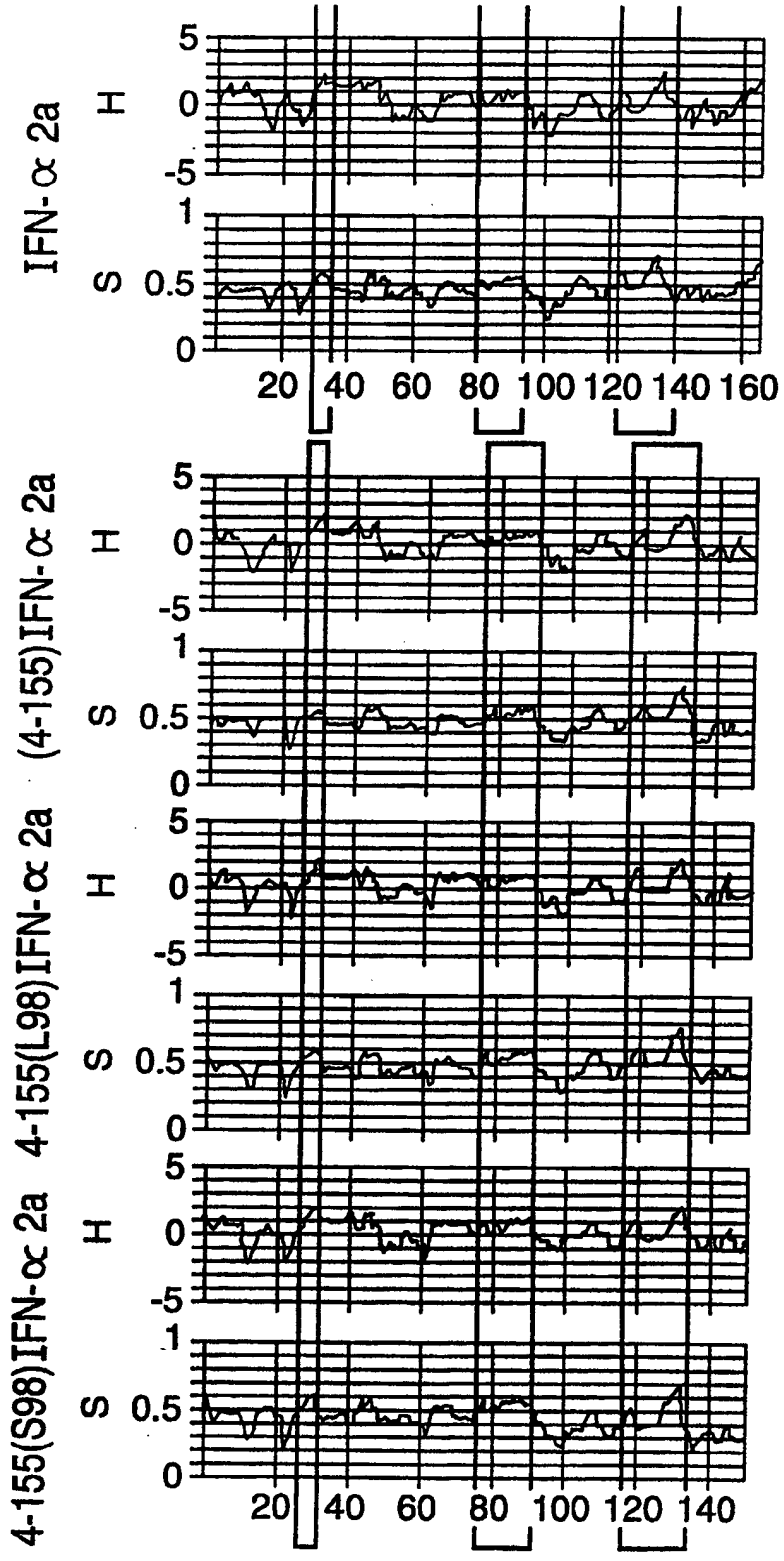
IFN-α 2a
(4-155)IFN-α 2a
4-155(L98)IFN-α 2a
4-155(S98)IFN-α 2a
H
S
5
0
-5
1
0.5
0
20 40 60 80 100 120 140 160
20 40 60 80 100 120 140

FIG.4A.

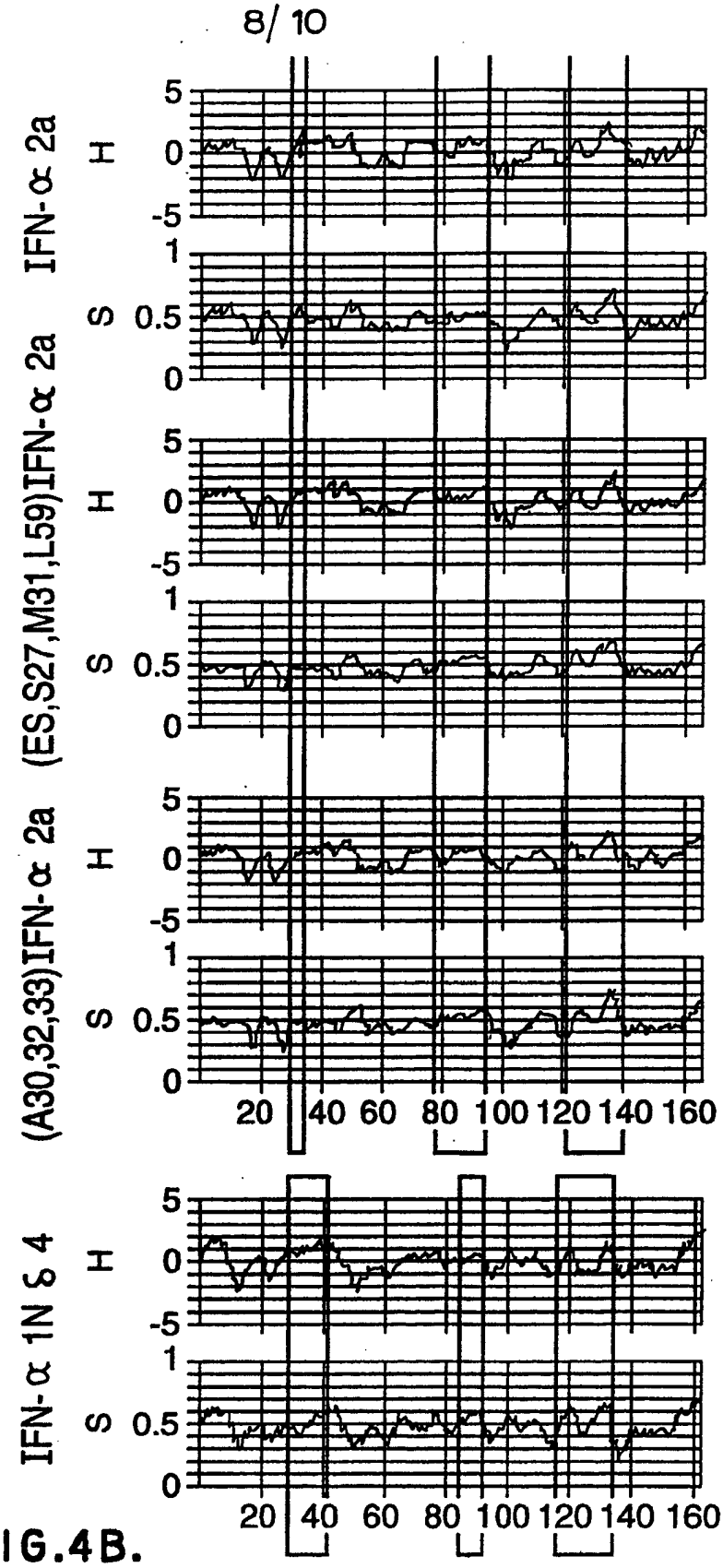
IFN-α 1N δ 4
(A30,32,33)IFN-α 2a
(ES,S27,M31,L59)IFN-α 2a
IFN-α 2a
H
S
5
0
-5
1
0.5
0
20 40 60 80 100 120 140 160

FIG.4B.

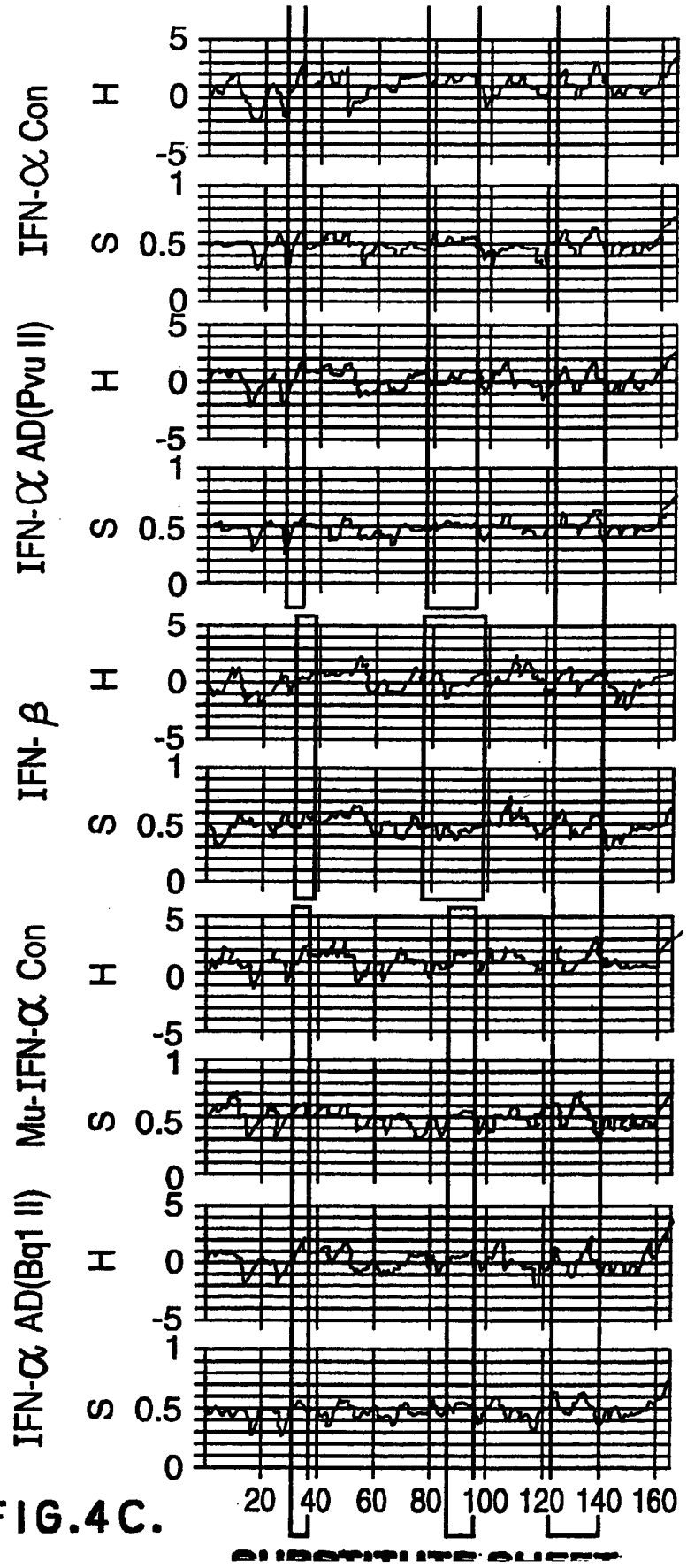
IFN-α Con
IFN-α AD(Pvu II)
IFN-β
Mu-IFN-α Con
IFN-α AD(Bq1 II)
H
S
5
0
-5
1
0.5
0
20 40 60 80 100 120 140 160

FIG.4C.

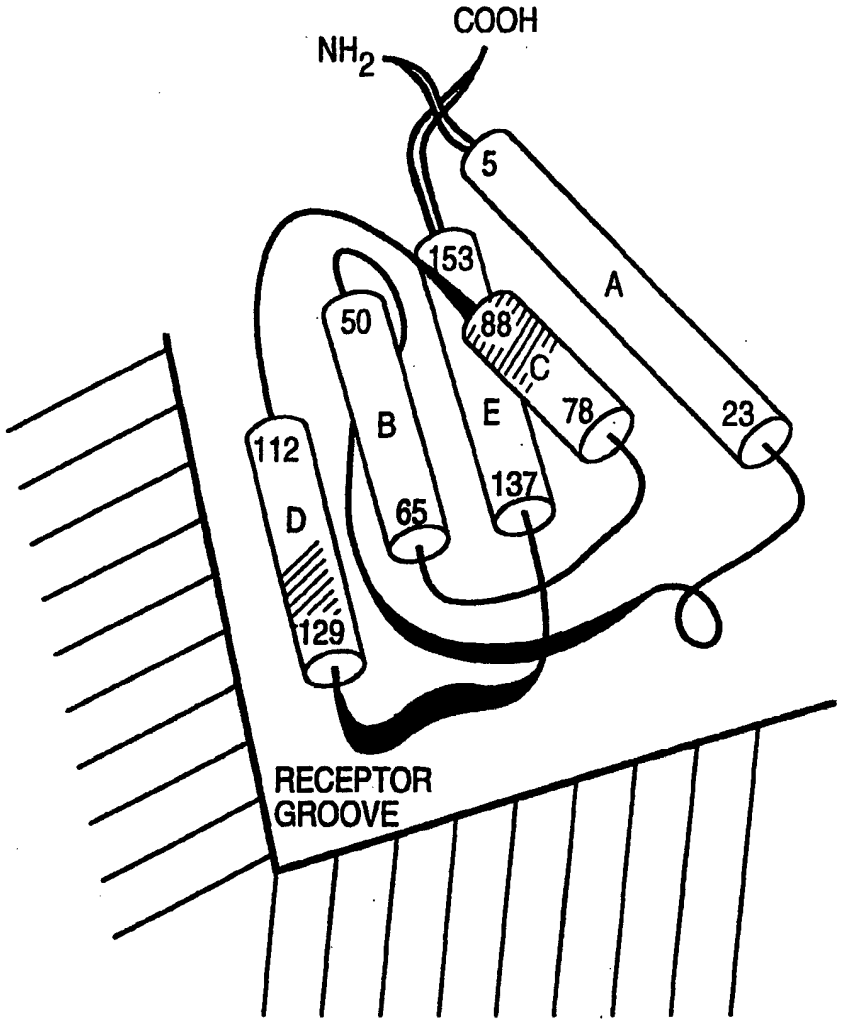

FIG.5.

INTERNATIONAL SEARCH REPORT

International Application No PCT/CA 93/00279

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07K7/06; C07K7/08; A61K47/42

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07K ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[10]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	JOURNAL OF INTERFERON RESEARCH vol. 9, no. 1, February 1989, pages 97 - 114 E.N. FISH ET AL 'the role of three domains in the biological activity of human interferon-alpha' cited in the application see the whole document ---	1-17
P,X	JOURNAL OF INTERFERON RESEARCH vol. 12, no. 4, August 1992, pages 257 - 266 E. N. FISH 'Definition of receptor binding domains oin interferon-alpha' see the whole document --- -/--	1-17

[10] Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12 OCTOBER 1993	15 -11- 1993
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	LE CORNEC N.D.R.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	THE BIOLOGY OF THE INTERFERON SYSTEM . E. DE MAEYER ET H. SHELLEKENS (EDS). 1985, AMSTERDAM : ELSEVIER. pages 157 - 162 HOROVITZ O. ET AL 'Two regions of the human IFN-alphaC molecule involved in binding to human cell receptor' * see the whole document especially page 161-162 *	1-7
A	NATURE. vol. 294, no. 5838, November 1981, LONDON GB pages 278 - 280 H. ARNHEITER ET AL 'Physicochemical and antigenic properties of synthetic fragments of human leukocyte interferon' cited in the application see the whole document	1-7
A	WO,A,9 203 144 (RESEARCH DEVELOPMENT FOUNDATION) 5 March 1992 see page 9, line 7 - line 13	1-17
A	JOURNAL OF BIOLOGICAL CHEMISTRY. vol. 262, no. 13, 5 May 1987, BALTIMORE US pages 6227 - 6237 A. SHAFFERMAN ET AL 'Specific residues within an amino-terminal domain of 35 residues of interferon-alpha are responsible for recognition of the human interferon-alpha cell receptor and for triggering biological effects' see the whole document	1-7

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

CA 9300279
SA 76346

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 12/10/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9203144	05-03-92	AU-A-	8624091	17-03-92
		CA-A-	2088068	15-02-92
		CN-A-	1058990	26-02-92
		EP-A-	0546087	16-06-93